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THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

THE HISTORY OF THE FERTILIZATION PROBLEM[1]

WE come together at this season of the year to discuss the latest advances in our science and to listen to the announcement of new discoveries. This implies a philosophy of life, an optimistic philosophy; we would not work as individuals nor assemble as societies if we did not believe that science is worth while, and that human progress is both possible, and, for some inscrutable reason, worth working for. This was the philosophy of science in the time of the Greeks, and it is the philosophy of our science of scarce four hundred years' growth. Modern science, I need hardly say, was entirely European in its origin, as is our American scientific population; and all science is ours to promote and advance by right of inheritance no less than of intellectual sympathy. Now that the great war is so largely arresting the progress of science in Europe it is our bounden duty to see that there is no halting in America; we should hold fast to our faith and strengthen our efforts for the advancement of science.

As we all labor for progress in science, I thought it would not be entirely out of place if, instead of dealing with some new subject, I attempted to lay before you a picture of the total progress in some central problem of biology; it can be nothing more than a sketch, but it may perhaps

[1] Address delivered before the American Society of Naturalists, and the Zoological Section of the American Association for the Advancement of Science, December 30, 1915.

help to justify our faith, and also to temper our conceit, if we have any. Fertilization is such a central problem which has interested mankind from the dawn of reasoning on account of its fundamental character, and is more or less interwoven with the thought of all ages.

Aristotle and Harvey.—It must be remembered in beginning our topic that the problem of fertilization was not clearly separated from the general problem of reproduction until well into the nineteenth century. In early human culture reproduction received its only interpretation at the hands of priests and mystery men; its first philosophical and scientific treatment was one of the great distinctions of the Greeks, especially of that great philosopher and father of science, Aristotle, who combined observation and reflection in the interpretation of nature. Aristotle devoted a separate treatise, which has come down to us, to animal reproduction. Among other things he studied the development of the chick day by day with so much detail that Harvey felt impelled to say, 1,900 years later:

> Aristotle among the ancients, and Hieronymus Fabricius of Aquapendente, among the moderns, have written with so much accuracy on the generation and formation of the chick from the egg that little seems left for us to do.

From the time of the Greeks to that of Harvey (1651) there was but little progress in the knowledge of reproduction, and none in the theory, as will appear from the views of Aristotle, the current views of medical men of Harvey's time, and of Harvey himself. Aristotle says:[2]

> The male is the efficient agent, and by the motion of his generative virtue (genitura), creates what is intended from the matter contained in the female; for the female always supplies the matter, the male the power of creation, and this it is which constitutes one male, another female. The body and the bulk, therefore, are necessarily supplied by the female; nothing of the kind is required from the male; for it is not even requisite that the instrument, nor the efficient agent itself, be present in the thing that is produced. The body then proceeds from the female, the vital principle (anima) from the male; for the essence of every body is its vital principle (anima).

With more common sense, if with less metaphysical subtlety, the physicians of the Middle Ages held, according to Harvey, that conception is due to a mingling of male and female seminal fluids,

> the mixture having from both equally the faculty of action and the force of matter; and according to the predominance of this or that geniture does the progeny turn out male or female (quoted from Harvey, Ex. 32).

Harvey's observations contained much that was new and significant, but the facts that he knew were inconsistent both with Aristotle's ideas and those of the physicians. They were, however, inadequate for sound generalization.

> Wandering between two worlds, one dead
> The other powerless to be born,

he descended deeper into the slough of metaphysics than Aristotle, and committed himself to the fantastic idea that conception in the uterus is identical with, or at least analogous to, conception in the brain; and that the ovum is the product of such unconscious uterine desire or conception, and receives no material substratum from the male![3] The theory of reproduction

[2] "De Gen. Anim.," lib. II., cap. 4, quoted from Harvey "On the Generation of Animals," Ex. 29.

[3] "Since there are no manifest signs of conception before the uterus begins to relax, and the white fluid or slender threads (like the spider's web) constituting the 'primordium' of the future 'conception' or ovum, shows itself; and since the substance of the uterus, when ready to conceive, is very like the structure of the brain, why should we not suppose that the function of both is similar, and that there is excited by coitus within the uterus a something identical with, or at least analogous to, an 'imagination' (phantasma) or a 'desire' (appetitus) in the brain, whence comes

was no whit more advanced in the middle of the seventeenth century than in the time of Aristotle.

The Period of Leeuwenhoek.—The use of the microscope in biological research began in the seventeenth century; it was the improvement of this new instrument of investigation and its application to the study of the reproductive substances that furnished the first fundamental advance in the theory of reproduction at the hands of Leeuwenhoek, viz., the discovery of the spermatozoa[4] in 1677.

This discovery aroused the greatest interest in scientific circles; a number of investigators repeated the observations and a spirit of speculation which led to wild flights of the imagination was aroused. Leeuwenhoek had soon to defend his priority in the matter and to protest against certain very imaginative views. Thus in a letter dated June 9, 1699,[5] he defends his priority and combats the notion that the human form can be observed in the spermatozoa. He inveighs especially against a certain Dr. Dalen Patius, who claimed to have seen the human form,

> the two naked thighs, the legs, the breast, both arms, etc., the skin being pulled up somewhat higher did cover the head like a cap.

Leeuwenhoek states that he can find nothing of the sort, but he adds:

> I put this down as a certain truth, that the shape of the human body is included in an animal of the masculine seed; but that a man's reason shall dive or penetrate into this mystery so far, that in anatomizing one of these animals of the masculine seed we should be able to discover the entire shape of the human body, I can not comprehend.

In a letter dated two weeks later he distinguishes two sorts of these animalcules, and concludes that the one sort is male and the other female.

the generation or procreation of the ovum. For the functions of both are termed 'conceptions,' and both, although the primary sources of every action throughout the body, are immaterial, the one of natural or organic, the other of animal actions; the one (viz., the uterus) the first cause and beginning of every action which conduces to the generation of the animal, the other (viz., the brain) of every action done for its preservation. And just as a 'desire' arises from a conception of the brain, and this conception springs from some external object of desire, so also from the male, as being the more perfect animal, and, as it were, the most natural object of desire, does the natural (organic) conception arise in the uterus, even as the animal conception does in the brain.

"From this desire or conception, it results that the female produces an offspring like the father. For just as we, from the conception of the 'form' or 'idea' in the brain, fashion in our works a form resembling it, so, in like manner, the 'idea' or 'form' of the father existing in the uterus generates an offspring like himself with the aid of the formative faculty, impressing, however, on its work its own immaterial 'form' " (from William Harvey, "On Conception," 1651).

[4] This discovery is sometimes credited to Hamm, described as a student of Leeuwenhoek's. The latter himself describes the occurrence as follows (*Phil. Trans.*, 1678, containing a letter from L. dated November, 1677): A certain Professor Cranen, who had frequently visited Leeuwenhoek for microscopical demonstration, requested by letter that he should give Dominus Hamm, a relative of his, some demonstrations of his observations. On his second visit D. Hamm brought in a glass vial some seminal fluid and stated that he had observed living animals in it; Leeuwenhoek confirmed this observation and repeated it many times. In this letter he gives a fair description of the spermatozoa, their form, size and movements and stated that he had observed them three or four years previously and mistaken them for globules. He did not at this time speculate as to the meaning of the spermatozoa, but in true scientific spirit began to make comparative observations, and in 1678 he described and figured spermatozoa of the rabbit and frog among others.

The credit of this discovery seems to me to belong rightly to the investigator whose wide experience in the field of microscopical anatomy and whose scientific acumen enabled him to grasp the possible significance of the discovery; not to the chance observer who called Leeuwenhoek's attention anew to the subject.

[5] *Phil. Trans.*, Vol. 21.

In France in the year of 1694, Nicholas Hartsoeker claimed to have been the first to have discovered the spermatozoa more than twenty years previously, although he did not publish until 1678, a year later than Leeuwenhoek's publication. Hartsoeker's ideas are characterized by a high degree of precision. He believes that each spermatozoon conceals beneath its "tender and delicate skin" a complete male or female animal, "which would perhaps appear if it could be seen like the following figure."[6] The egg is merely a source of nourishment for the real germ contained in the spermatozoon. In birds the spermatozoon enters an egg to be nourished; there is but a single opening in the egg, situated over the so-called germ, and this opening closes after a single spermatozoon is admitted; but if two spermatozoa enter they unite and form a double monster. In mammals the tail of the spermatozoon is the umbilical cord; this unites with the ovum, *i. e.*, the placenta, and the latter with the uterus. Each one of male animals (spermatozoa) encloses an infinity of other animals both male and female, which are correspondingly small, and those male animals enclose yet other males and females of the same species, and so forth in a series which includes all the members of the species which are to be produced up to the end of time. No difficulty was found in this conception, for the atomic theory of matter was not yet placed on a scientific basis.

Thus was founded and flourished for its brief day the school of the spermatists. Unhampered by any scientific conception of matter, living or non-living, there was no obstacle to the eye of faith and no impediment to the age-old longing to make an intelligible universe out of the scraps of experience.

[6] The figure in question is reproduced in Kellicott's General Embryology, p. 22.

The Period of Spallanzani.—In the entire eighteenth century, although speculation continued rife, there was only one notable contribution to our subject. This was the work of the Abbé Spallanzani, "Expériences pour servir à l'histoire de la génération des Animaux et des Plantes," published in Geneva in 1785. His working hypotheses were naturally in the spirit of the times. Theories of reproduction, he says, may be reduced to two.

> The one explains the development of organisms mechanically, the other supposes them to preexist, and waiting only for fertilization to develop them. The second system has given birth to two different parties, one believing that the organism is preformed in the ovum, the other that it is preformed in the spermatozoon.

Spallanzani believed that his observations destroyed the epigenetic theory as propounded by Buffon and others, because they demonstrated the existence of the fetuses (ova) in the females of toads, frogs and salamanders, prior to the act of fertilization, which according to the epigenesists animates or creates the germ. For the same reason the spermatists must also be wrong. Spallanzani thus combated epigenesis as understood in the eighteenth century, and also the ideas of the spermatists, and he was led to deny that spermatozoa are necessary for fertilization, and to hold that the fertilizing power of the seminal fluid resides not in the spermatozoa, but in the fluid medium that accompanies them; and this in spite of the fact that his final experiments really proved the reverse.

His work contains a great wealth of observation and experiment, so that it will be possible merely to indicate some of his chief results. In the first place he demonstrated that in frogs and toads fertilization takes place outside of the body, and for the first time he successfully carried out artificial insemination, thus laying the foundation for the artificial propagation of many ani-

mals. In making these experiments he thought he found cases in which seminal fluid devoid of spermatozoa would fertilize and thus fell into the error, which he was so ready to accept from his opposition to the spermatists, that the fluid medium of the seminal fluid was the fertilizing substance. He also investigated the conditions of successful insemination, with reference to the duration of fertilizing power, exposure to various chemicals, to heat, etc. The amount of dilution of which the seminal fluid was capable was also carefully investigated. By experiment he excluded the idea that fertilization might be an effect of an emanation, or vapor arising from the sperm.

He concluded that the seminal fluid acted by accelerating the vital processes; it enters the body through pores, and stimulates the action of the heart. This idea offered no difficulty to one who believed that the organism was preformed in the ovum, and it was supported by the observation that the beating of the heart was the first observable movement of the embryo. Bonnet suggested to him the problem, if the spermatic fluid might stimulate the heart of the embryo in the process of fertilization, why might not other fluids produce the same effect? He was thus led to attempt the first experiments on artificial parthenogenesis; he tried to start the development of eggs by electricity, by the action of extracts of all the various organs, by vinegar, dilute alcohol, lemon juice, and other substances, all without effect.

It is interesting to see how his experiments led to hypotheses and these, even though wrong, to further experiments, some of which, like his experiments on artificial parthenogenesis, were not taken up again in a fruitful way for over a century.

His final experiments are those so often quoted as furnishing the proof that fertilizing power resides in the spermatozoon. He showed that, if diluted sperm be filtered through a sufficient number of layers of filter paper, the filtrate has no fertilizing power, whereas the residue washed off the filter paper will fertilize. But he did not himself draw the correct conclusion; he says the experiment proves "that filtration removes from spermatized water its fertilizing power, inasmuch as the seminal fluid which was contained in it remains on the filter papers, from which one can extract it by pressing them." It is perfectly clear that Spallanzani himself never held that the spermatozoa themselves were the fertilizing agents, but, on the contrary, he contests this idea strongly as leading to spermatist delusions.

1800–1870.—After Spallanzani there was no real advance in the theory of fertilization until the publication of Prevost et Dumas' "New Theory of Reproduction" in 1824. They observed that young animals incapable of breeding, old animals beyond the breeding stage, the infertile mule, and birds outside of the breeding season possess no spermatozoa, and they conclude that these facts "sufficiently prove the importance of the animalcules, and show that there exists an intimate relationship between their presence in the reproductive organs and the fertilizing power of the animal." In a long series of experiments they investigated the conditions of fertilization in frogs: all conditions that destroy the animalcules destroy also the fertilizing power of sperm suspensions; the filtrate of a sperm suspension devoid of spermatozoa will not fertilize; the redissolved residue of a suspension evaporated to dryness will not fertilize, etc.; the number of eggs fertilized is always less than the number of animalcules employed. So that they came to the conclusion that "the prolific principle resides in the spermatic animalcules."

In their subsequent publications they concluded that it is

infinitely probable that the number of animalcules employed in fertilization corresponds to that of the embryos developed . . . so that the action of these animalcules which we regard as the male reproductive elements is individual, not collective.

They concluded that a spermatozoon penetrates each egg and becomes "the rudiment of the nervous system, and that the membrane (germ disc of the egg) in which it is implanted, furnishes, by the diverse modifications which it undergoes, all the other organs of the embryo."

These studies gave a new impetus to the study of fertilization; some were convinced that Prevost et Dumas were essentially correct, while others still adhered to the idea that the fluid part of the seminal fluid was the fertilizing medium. Thus the celebrated embryologist Bischoff in 1842 does not hesitate to declare outright for the latter view "that only the dissolved part of the semen penetrates into the egg and thus completes fertilization." He considered that

Valentin's hypothesis united all the facts; the seminal fluid is so unstable chemically as to break down as soon as the particles come to rest; it is similar to the blood in this respect, but it is not in regular circulation and the function of maintaining its chemical composition is relegated to the movements of the spermatozoa.

However, Bischoff subsequently became convinced that the spermatozoa were themselves the essential agents, though he still refused to believe in the penetration of the egg. Kölliker had put forward a contact theory of fertilization, which Bischoff regarded merely as a statement of facts requiring further development. He therefore adopted the idea of catalyzers, at that time a new idea in chemistry, and held that the spermatozoon was essentially a catalytic agent, *i. e.*, as he defined it, "a form of matter characterized by definite transformation and internal movement" which it transmits by contact to the egg, which is in a condition of maximum tension or inclination to assume the same form of transformation and movement. Fertilization is thus not a process of union and fusion as in ordinary chemical combination, but a catalytic process, as defined above.

This point of view deserves to be emphasized as one of the first attempts at a physico-chemical explanation of fertilization.

For some time naturalists were divided between the two points of view, viz., that of Prevost et Dumas, that the sperm penetrated into the egg, and that of Kölliker and Bischoff that it acted by contact. Lallemand (1841) well expresses the view of those who believed in the union of the ovum and spermatozoon:

Each of the sexes furnishes material already organized and living. . . . A fluid obviously can not transmit form and life which it does not possess. . . . Fertilization is the union of two living parts which mutually complete each other and develop in common. . . . When one embraces in a single point of view the reproduction of all living beings, one arrives at the following more general formula: Reproduction is the separation of a living part which may either develop separately or acquire from another living part the supplementary elements necessary for the ulterior development of a being similar to the type. . . . The preservation of the type is due to the extension of the same act which has produced the development of each individual being.

This is the most complete statement of the principle of genetic continuity that I have found in the literature of this period.

These observations and conclusions were found on the eve and early morrow of the greatest biological generalization, the cell-theory. Though Schwann interpreted the ovum as a cell (1838), this view did not at once become dominant, and was generally accepted only after over twenty years of discussion. The view that spermatozoa were parasitic organisms was more or less

current until Kölliker in 1841 showed by their development that they were modified cells. Nevertheless, there was, strictly speaking, no immediate application of these results to the problems of fertilization.

The half century from 1824 to 1874 yielded relatively little advance in fertilization theory; the opinion that the spermatozoon actually penetrated into the ovum gradually gained ground largely from the very logic of the situation, but partly from various observations. Bischoff's contact theory, which was the only alternative, was criticized because if the sperm does not penetrate, but remains outside of the membrane, there is absence of that direct contact between sperm and egg substance postulated by the theory. Wagner's criticism was also very effective; a ferment does not determine the character of a reaction, but the spermatozoon does, for it transmits paternal characteristics. In the way of observations Barry in 1840, Newport,[7] 1854–1855, Meissner, 1855, and others maintained observations of penetration of the ovum by the spermatozoon; Keber (1854) laid especial emphasis on the micropyle as adapted for entrance of a spermatozoon. These observations were on the whole inconclusive, for actual penetration was not observed, but inferred from the presence of spermatozoa inside the egg membrane. Moreover, the spermatozoon could not be discovered within the egg.

The Modern Period.—The preceding period (1824–1874) was coincident, as we have seen, with the early history of the cell theory, but the demonstration of the unicellular character of the ovum and spermatozoon had little effect upon the problems of fertilization. The cell theory was still incomplete; the free formation of the nuclei was still held by competent naturalists, and nothing was known of the phenomena of karyokinesis. The cytological investigations of the next ten years (1874–1884) were destined to lay the foundations of the modern nuclear theory in its broad outlines. The fertilization studies of this period were mainly morphological, and while it is correct to say that they were largely dominated by the growing nuclear theory, it is also strictly true that they contributed in no small measure to its upbuilding. Though the penetration of the spermatozoon into the egg had long been suspected, it was first clearly demonstrated in this time; the origin of the egg nucleus by two successive divisions of the germinal vesicle was discovered; the origin of the sperm nucleus from the head of the spermatozoon, the sperm aster, the union of the egg nucleus and the sperm nucleus, the relation of these to the first cleavage spindle, the origin of the fertilization membrane, the ill effects of polyspermy and the theory of its prevention; and finally the doctrine of the equivalence of the egg and sperm nuclei, and the biparental character of the nuclei of sexually produced organisms, as first laid down by Van Beneden, were products of the period also. No period of cytological research seems to me of greater significance than this.

There was almost a complete cessation of investigation from 1855–1873, when the dawn of the modern period broke suddenly. In 1873 Bütschli observed in the egg of a nematode the approach and contact of the two structures, which we now know to be the germ-nuclei, immediately preceding the first cleavage of the ovum. But no interpretation was presented. In 1874 Auer-

[7] Newport's observations rose to a higher plane than those of the others, for he actually observed in the frog's egg (1) that the first plane of cleavage is in line with the point on the egg artificially impregnated, (2) that it marks the plane of symmetry of the embryo, (3) that the head of the young frog is turned towards the same point.

bach[8] described the appearance of two nuclei at opposite ends of the elongated egg of Rhabdites; these increase in size, migrate towards the center of the egg, meet, rotate through 90° and fuse together. A dicentric figure appears and cleavage follows. What is the origin of these two nuclei and the significance of their union? The fusion of two nuclei was at the time entirely without analogy. Auerbach states:

> It is natural to assume that, as for the reproduction of organisms the copulation of two individuals, or at least of two cells in some form or other is so frequently necessary, so here a similar condition is found for nuclear reproduction.

Auerbach supposes the two nuclei which appear at opposite ends of the elongated egg to have arisen freely; one of these comes from the end where the spermatozoa had penetrated, the other from the opposite end where the germinal vesicle had disappeared. The difference of the origin influences the quality of the nuclear materials arising *de novo;* fusion of the nuclei counteracts the differences thus arising; but all this would be undone if the division of the fusion nucleus followed along the plane of the union; hence the rotation through 90°.

In the next year Bütschli again observed fusion of nuclei in nematode eggs before the first cleavage. However, he did not accept Auerbach's interpretation, but he tended to regard it as a general law of nuclear formation, that first two or several small nuclei arise and subsequently fuse; this he finds to occur even in the blastomeres of the four- and eight-cell stages.

About the same time (1875) Van Beneden also observed similar phenomena in the rabbit's egg. He did not see spermatozoa enter the egg, but he found them with their heads closely applied to the surface in every unsegmented egg, and came to the conclusion that fertilization consisted essentially in fusion of the spermatic substance with the superficial layer of the vitellus. At a little later stage he found a small nucleus in the cortical layer of the egg; this he called the peripheral pronucleus; a central pronucleus appeared simultaneously. They grow, approach one another and meet in the center. Later there is only one nucleus, probably formed by the union of the two.

> As I have shown that the spermatozoa attach to the surface of the vitellus and mix with its superficial layer, it appears probable to me that the superficial pronucleus is formed, partially at least, at the expense of the spermatic substance. If, as I think, the central pronucleus is constituted of elements furnished by the egg, the first nucleus of the embryo would be the result of union of male and female elements. I put forth this latter idea simply as a hypothesis, an interpretation which may or may not be accepted.

The way was now clear for the definitive solution of the old riddle of the relation of the egg and spermatozoon, which was quickly furnished by O. Hertwig and Hermann Fol. The observations of these authors appear to have been made independently and nearly simultaneously. In 1875 Hertwig observed and described correctly the principal phenomena of fertilization in the sea-urchin egg. He did not actually see the penetration of the spermatozoon, but he observed the sperm nucleus and its aster so soon after that he had no doubt of the correct interpretation; he also observed the approach of the sperm-nucleus and the egg-nucleus to the center of the egg and their apparent fusion.

> Fertilization has been previously interpreted as a fusion of two cells, but we have now seen that the most important process involved is the fusion of the two nuclei. The union of the egg-nucleus with the sperm-nucleus is necessary to produce a nucleus endowed with living forces adequate effectively to stimulate the later developmental processes in the yolk, and to control them in many respects.

Fol's observations, made partly independently of Hertwig's and partly after the

[8] "Organologische Studien."

publication of Hertwig's first paper, supplemented Hertwig's in several important respects: (1) He observed the details of penetration of the spermatozoon with a clearness that has never been surpassed for these forms. (2) He gave the first correct account of the maturation divisions and origin of the egg-nucleus (Hertwig regarded the latter as being the persistent nucleus of the germinal vesicle). (3) He paid special attention to the origin of the fertilization membrane and founded the classic theory that it was an adaptation to prevent polyspermy. (4) He was the first one adequately to present the harmful effects of polyspermy.

The period initiated by these two men was characterized mainly by the repeated demonstration of penetration of the spermatozoon, the formation of a nucleus from the sperm head, and the fusion of this nucleus with the egg-nucleus. It was also gradually demonstrated that the egg-nucleus is genetically derived from the germinal vesicle by karyokinetic divisions. Thus the genetic continuity of the germ nuclei with nuclei of preceding cell generations was established. As yet the character of the fusion of egg and sperm nuclei had hardly been raised, for the chromosome problems and hypotheses were in a very nascent state. Flemming's discoveries concerning chromosomes and their reproduction in karyokinesis by splitting date only from 1876–1878.

All the problems of cell morphology were in a fine state of fermentation during this time, the really classic period of cell-morphology; the foundations of our present knowledge of cell-division were being laid; before the decade 1870–1880 it had been firmly established that cells arise only by division from preexisting cells; but two views of the origin of nuclei were still held, one that of free formation, according to which the nuclei of daughter cells had no genetic connection with the nucleus of the mother cell, and the other that nuclei arise by division from a preceding nucleus. Little by little as a result of numerous investigations by many investigators, both zoologists and botanists, the matter cleared up. In 1878 Flemming was able to outline the whole scheme of karyokinesis substantially as we now understand it.

The fundamental biological principle of genetic continuity was foreshadowed by the founders of the cell doctrine, and was more or less distinctly foreseen by some of their contemporaries, as in the case of Lallemand. It was yet more clearly expressed in Virchow's famous aphorism, *omnis cellula e cellula* (1856); but it could not become an established guiding principle in genetic research until the entire cell-cycle of the individual life history was worked out in broad outline, until the process of cell division was accurately ascertained and applied to the genealogy of the germ-cells, until the respective parts of ovum and spermatozoon in the origin of the new generation were understood, nor until the hoary doctrine of spontaneous generation was banished bodily from the field of biology. These were all accomplishments of that great decade in biological research, 1870–1880, for which the studies of the preceding thirty years had furnished ample preparation. The entire superstructure of modern genetic research rests upon the foundations then laid.

Professor Mark's paper on *Limax* (1881) is a point of departure between the fertilization studies of the seventies and those that were to follow. Professor Mark observed that the pronuclei come together, but do not fuse to form a first cleavage nucleus, as had been described for other animals.

The first cleavage nucleus does not have a morphological existence.

The pronuclei persist after the appearance of the cleavage centers, their membranes then gradually disappear.

In 1883 van Beneden published his now classic paper on *Ascaris*: The pronuclei do not unite, both are included in a single amphiaster; each produces two chromosomes; these divide and their halves form the daughter nuclei. In the nuclei of the first two cells there are thus equal numbers of male and female elements, and there are reasons to believe that even in these two nuclei they do not fuse; it is probable that they remain distinct in all derivative cells, including the immature eggs and spermatogonia. In the egg the chromatin is composed of two distinct parts, and

it is legitimate to suppose that each is the equivalent of a male and a female chromosome, and that in the formation of the polar globules each throws out the male chromatin which it contains.

Van Beneden by a veritable stroke of genius thus anticipates the entire chromosome doctrine of the present time, even though certain aspects of his interpretation were not entirely fortunate: his conception of the diploid cells as hermaphrodite, for instance, and freeing of egg and sperm from the male or female elements in maturation.

With the establishment of the nuclear theory, destined soon to be elevated into the doctrine of chromosome individuality, a certain duality of cell-life was recognized in which nucleus and cytoplasm, however interdependent, were regarded as playing specific rôles. But there was no logical reason for stopping at duality, and the centrosome soon came to be recognized under van Beneden and Boveri's leadership as a third organ of cell-life reproducing itself by division. The development of this idea was due not only to studies of karyokinesis, but also to the series of fertilization studies which began with Boveri's classic papers on *Ascaris* (1887–1888). In these papers Boveri is convinced of the necessity of making "the sharpest distinction between fertilization and heredity, *i. e.*, between the question how egg and spermatozoon produce a cell capable of division, and the question how these cells come to be capable of reproducing the qualities of the parents in the offspring"; this distinction has since been generally recognized. Boveri's solution of the fertilization problem was in terms of the centrosome hypothesis: the egg is devoid of the organ of cell-division, the centrosome; capacity for division, hence the initiation of the developmental processes, is restored through the introduction of a centrosome into the egg by the spermatozoon.

This conception exerted a dominating influence on the series of fertilization studies which followed; the questions as to the origin of the sperm aster with its contained centrosome in the egg, and as to the genetic continuity of the sperm centrosome with the centrosomes of the cleavage amphiaster were energetically investigated by a series of students for the next fifteen years or more, and similar studies have continued with less energy down to the present time. Collectively these publications constitute a fairly exhaustive record of the morphology of the fertilization process in animals, a large part of which was furnished by American students.

The morphological analysis of fertilization seems now to be fairly complete; there may still be disturbances such as recent attempts to trace mitochondria back to the sperm, which seems destined to share the adverse fate of the similar attempt to trace the centrosomes to the sperm; but there is not likely to be any great modification of the existing data, which seem to me to demonstrate, effectively if not absolutely, that the sperm head contains all the substances necessary for fertilization. We have thus

attained a more or less definitive solution of the morphological relations of egg and spermatozoon in the fertilization process.

The cytologist working with chromosomes and the geneticist with Mendelian factors have traced maternal and paternal elements through the life history in a manner very satisfactory to certain classes of biologists, however repugnant to others, so that we are beginning to see how certain strands of the web of life cross the gap of successive generations. It remains for the biology of the future to elucidate the chemical foundations of chromosome behavior and to identify the Mendelian factors in these chemical foundations.

The problems of the immediate reaction of the reproductive elements and the physiology of fertilization are not touched by this morphological analysis, though they had been present in the minds of investigators from the beginning. The experimental investigation of these problems dates from Spallanzani, as we have seen, but they did not become dominant until the morphological problems of fertilization were in an advanced stage of solution. They constitute, however, the more immediate problems of fertilization, considered in a restricted sense.

We have had two lines of attack since the studies of O. and R. Hertwig published in 1887 really initiated the modern period in the physiology of fertilization. The one is a direct experimental analysis of the fertilization process itself; the other is the attempt to imitate the action of the spermatozoon by chemical and physical agencies, in short the studies on artificial parthenogenesis. I shall not attempt to deal with the latter, which constitute one of the most interesting and suggestive chapters in modern biology, beyond attempting to define their relation to the problems of fertilization proper.

It was soon found in the course of studies on artificial parthenogenesis that no single physical or chemical agency suffices to initiate development in all eggs, and that when the various agencies effective in all the successful experiments are assembled they constitute a fairly complete list of agencies to which protoplasm in general is irritable. The idea then arose that the common factor must be the effective one, but no common factor has been found, or can be found, in the agents themselves; the only common factors are in the reproductive cells. This leaves the method of parthenogenesis in the same position as the method of analysis, that is in the position of determining what are the changes in the egg itself that initiate development, and what is the nature of their dependency upon the external agent or spermatozoon? The answer to these questions can not proceed exclusively from parthenogenetic studies, though to the extent that the same questions are involved parthenogenesis and fertilization studies must furnish the same answer. But there are obviously fundamental problems of fertilization that can not be touched by methods of artificial parthenogenesis.

The conditions to be fulfilled in fertilization involve not only penetration of the spermatozoon, or some part of it, into the egg, but also reaction between the two, which is evidenced by the behavior of both partners; for it is possible for a spermatozoon to penetrate an egg and no reaction to be evidenced in the behavior of either the egg or sperm; as when immature eggs are penetrated by mature spermatozoa. We may therefore speak of a *fertilization reaction* when the behavior of both partners indicates that the process is proceeding normally. Fertilization has its quantitative aspect, and the reaction may be complete or exhibit varying degrees of incomplete-

ness. For a normal fertilization reaction certain internal conditions of the partners and certain external conditions of the medium must be realized. The study of the external conditions throws light upon the reaction, because the nature of the internal conditions may be inferred from the necessary, from the inhibiting, and from the favoring conditions of the medium.

External Factors.—The fertilization reaction like all biological reactions requires certain conditions of the environment, such as definite range of temperature and chemical composition of the medium. In the first place, if these are exceeded in either direction so far as to injure the cells the fertilization reaction either does not take place, or it is rendered abnormal. The cause of the failure, or the abnormality, in such cases lies in some change of the internal composition of one or the other of the germ-cells. The classic experiments of this kind are those of Oskar and Richard Hertwig published in 1887. These investigators studied the effects of high temperature, various injurious chemical reagents and of mechanical shock on the germ-cells separately before fertilization, and on the process of fertilization itself at various stages. Many exceedingly interesting observations were made, and problems were raised that were not then ripe for solution. Other experiments of a similar kind have since been made, but their consideration properly belongs to the problems of the internal factors, for the phenomena observed depend upon internal changes of the germ-cells.

In the second place there may be modifications of the medium which do not directly injure the germ-cells, but which inhibit or favor the fertilization reaction. Examples of inhibiting phenomena are found in Professor Loeb's studies of the relations of ions to the fertilization reaction, or my own on the inhibiting action of blood or tissue secretions of the same species on fertilization. The most striking example of conditions favoring fertilization is the action of alkalis in enabling interclass hybridization, discovered by Jacques Loeb. Such experiments furnish important data for the analysis of the reaction, but it is obvious that their interpretation must depend upon internal conditions of the fertilization reaction.

In the third place the membranes of the egg and of the spermatozoon must influence the occurrence, rate and extent of the fertilization reaction according to the degree of their permeability to the substances concerned; the egg-membrane is of course more especially concerned; its rôle in the occurrence of parthenogenesis has been studied especially by R. S. Lillie; and I have found in the case of the starfish egg that a resistant egg-membrane may entirely block the fertilization reaction, though the block may be removed by agents that render the membrane more permeable.

The internal conditions of the fertilization reaction may be grouped under two heads: (1) Maturity of the germ-cells; (2) specificity of the reaction.

1. *Maturity.*—Concerning conditions of maturity of the spermatozoon but little definite is known, except that it will not fertilize before its differentiation is complete. Whether the cause of this lies entirely in deficient motility, or partly also in incomplete chemical differentiation, we do not know; though there are some reasons for thinking that the latter factor may be involved. In the case of the ovum our knowledge is in a much more advanced stage. We know that the fertilizable condition, which represents the final maturity of the ovum, arises rather suddenly, usually lasts but a short time, and is lost as an immediate consequence of the fertilization reaction. (*a*)

That the fertilizable condition arises suddenly has been shown especially by the work of Délage on the starfish egg and of Wilson on the egg of *Cerebratulus*. Their experiments on merogony showed that parts of the full-grown ovum taken prior to the rupture of the germinal vesicle are incapable of fertilization; but, soon after the rupture of the germinal vesicle, parts, whether nucleated or not, readily fertilize. Hertwig's observations (1877) also showed a complete failure of the fertilization reaction in primary ovocytes of the sea-urchin before rupture of the germinal vesicle, even when spermatozoa penetrated. I have observed the same thing in *Chætopterus*.

(*b*) Eggs of *Platynereis* lose their capacity for fertilization almost immediately after coming into sea water, even though spermatozoa may penetrate (Just); eggs of the frog become unfertilizable after half an hour in water (Spallanzani); eggs of the wall-eyed pike completely lose their fertilizability after ten minutes in water (Reighard). Usually fertilization capacity begins to fall off in one or two hours after eggs are laid in most marine animals, though in some, as in the sea-urchin, it may persist much longer.

(*c*) Once fertilized eggs do not fertilize again, nor do parts of such eggs freed of the fertilization membrane. It should therefore be impossible to superimpose parthenogenesis and fertilization; and the studies of Mr. C. R. Moore, one of my students (not yet published), show this to be the case. Apparent superposition appears in all cases to be due to incomplete reactions, which cease and may be subsequently resumed. The fertilization reaction appears to be irreversible; and the appearance of reversal in parthenogenesis may be referred, like superposition of fertilization on parthenogenesis, to incompleteness of the initial reaction.

Specificity is an outstanding feature of the fertilization reaction, the significance of which is not weakened by any hybridization experiments. We need not stop to define the limits nor the consequences of hybridization in order to justify the assertion that no theory of fertilization which fails to include the factor of specificity as one of the prime elements can be true.

The fundamental character of specificity is illuminated by the phenomena of self-sterility; in species where this occurs the eggs and sperm of the same individual are sterile *inter se,* though fertile with those of all other individuals. This has led some botanists to the conception of individual stuffs; but Correns's experimental analysis led him to the conclusion that the specific factor is not an individual stuff, but a definite combination of stuffs for each individual. The combination arises always with the individual, and disappears with it. An extension of the principle of self-sterility is found in that mutant of fruit flies discovered by Morgan in which the males and females are fertile with other mutants, but sterile *inter se.* The only biological parallel of such phenomena is found in the individual blood composition revealed by serological studies. That there is a common factor in species and individual specificity studies no one who has studied both sets of phenomena can doubt.

A consistent theory of fertilization must take account of all these phenomena, not only the internal factors of maturity of germ-cells and the specificity of their reactions, but also the external factors that favor or inhibit the reaction. I have attempted to show in a series of papers that the fertilizable condition of the egg depends upon the presence of a specific substance which is produced at the time of rupture of the germinal vesicle and which disappears completely after fertilization.

If this substance be present in the egg in adequate amount, the egg can be fertilized, otherwise not. It may be obtained in solution in the sea-water and recognized by its capacity for agglutinating sperm suspensions of the same species, in some cases at least. If it is thus possible to associate the fertilizable condition of the ovum with a definite substance, we have a base from which an analysis of fertilization can be made.

If the existence of such a substance be admitted, it must operate either by activating some substance in the spermatozoon, which is to be regarded as the effective agent in subsequent changes, or we must regard it as the effective agent which is transformed from an inactive to an active state by some substance in the spermatozoon. If we take the first alternative, we have no explanation of parthenogenesis, whereas if we regard the egg substance as the active agent, the explanation of parthenogenesis proceeds along the same lines as that of fertilization. Moreover, I have been able to show by an analysis of the phenomenon of blood inhibition of fertilization, that the first point of view is untenable.

This substance may therefore be called the fertilizing substance, or fertilizin. By its reaction it is shown to be a colloidal substance, not giving the usual protein tests, and exhibiting some of the properties of a ferment as shown by Richards and Woodward. Fertilization would thus be a three-body reaction between the sperm-receptors, fertilizin and egg-receptors linked in line; and it is possible to show that inhibiting agencies may operate at the various linkages of such a reaction. In its reaction with the sperm the fertilizin of different species exhibits a certain degree of specificity, which should be more fully studied, but which has been partly explored by Jacques Loeb and myself.

This form of hypothesis takes into account the internal factors both of maturity of the germ-cells and their specificity; it is also adapted to explain the environmental conditions of fertilization extremely well; and it is consistent with the results of parthenogenesis, and the known relations of parthenogenesis and fertilization to the permeable or impermeable conditions of the egg-membrane.

I believe that I speak for all naturalists when I express my admiration for the advances in the conception of the cell due to the labors of many physiologists. But those of us who deal with the more complex phenomena of cell-life as shown in fertilization, in the behavior of chromosomes, and in the phenomena of heredity, feel that no advance in our comprehension of the cell-membrane, of the relation of cell-activity to electrolytes, nor of the chemical analysis of triturated cells, will lead to a fundamental comprehension of these phenomena. There is a great gap in our knowledge of cellular physiology, the significance of which is not generally appreciated. We know nothing except what our microscopes show us, of the reactions of the colloidal substances of the living cells; and all hope of a physico-chemical analysis of cell activities is premature until the gap is filled in.

The main physiological problems of fertilization are still before us; all the work up to the present has merely prepared the way for their solution. Fertilization is the knot in the webs of successive generations which must be untied before we can trace the strands from generation to generation. The task bespeaks all that we know, or may hope to know, of cellular physiology. As in all times of the history of the subject our vision is limited by our general biological conceptions, and the problem will move forward as our general knowledge of

cell-life progresses; and it will aid in its turn in the general advance.

We have followed the history of the problem of fertilization from the metaphysical stage through the morphological stage into the physiological stage, and within sight of the physico-chemical stage. Possibly the results seem slight as a record of 265 years of continuous study of a single biological problem. But we read the history of science very superficially indeed if we fail to realize the constant interdependence of all scientific thought. There has probably been no time in the history of our particular subject when a greater amount of work on its problems would have caused a much more rapid advance. Scientific discovery is a truly epigenetic process in which the germs of thought develop in the total environment of knowledge. Investigation of particular problems can not be accelerated beyond well-defined limits; progress in each depends on the movement of the whole of science.

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THE WORK AND OPPORTUNITIES OF A DEPARTMENT OF RESEARCH MEDICINE IN THE UNIVERSITY [1]

If we analyze the discussions of present-day problems of medical education we find that an important if not the ultimate object of any particular plan is greater opportunity for research. This we find in the argument of those who support the plan of the full-time teacher, the plan that the university should own its hospital or control one by close affiliation, and also it is evident in all plans for greater endowment. Increased facilities for research and an augmentation of the number of men engaged in research, or combining research with teaching, would ensure, it is contended, not only important progress in the science of medicine, but also a higher development of both medical teaching and medical practise. To what extent this increased interest in research is due to the popularization of medicine through the practical application of discoveries in the fields of bacteriology and protozoology and to what extent to a dissatisfaction with time-honored methods in medical education, it is difficult to say. Both undoubtedly have had some influence but they alone can not explain the rapidly increasing number of experiments in medical education which have for their avowed object the stimulation of medical research in school and hospital. As the most important of such experiments I need only remind you of the so-called "full time" scheme at Johns Hopkins Medical School fostered by the General Education Board, the affiliation between Columbia University and the Presbyterian Hospital of New York City, the development in Chicago of the Otho S. A. Sprague Institute which, without buildings of its own, utilizes for purposes of research in medicine the already existing laboratories and hospitals of that city, and more recently in San Francisco in connection with the University of California, the establishment of a well-endowed department for general research in medicine. On a smaller scale we find the establishment, definitely within the university, of separate departments for the investigation of tropical diseases, of cancer, of tuberculosis, of chronic diseases, or of departments devoted less specifically to experimental medicine, comparative pathology, comparative physiology and the like. As all such foundations must be considered for a time at

[1] Address of the vice-president and chairman of Section of Physiology and Experimental Medicine of the American Association for the Advancement of Science, Columbus, January 1, 1916.